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L11: Entry 1 of 4 File: PGPB Oct 24, 2002

DOCUMENT-IDENTIFIER: US 20020155114 A1
TITLE: THERAPEUTIC MONOCLONAL ANTIBODIES THAT NEUTRALIZE BOTULINUM NEUROTOXINS

Detail Description Paragraph:
[0230] Affinity, binding kinetics, and in vitro toxin neutralization were determined for one representative scFv binding to each epitope. For each epitope, the scFv chosen for further study had the best combination of high expression level and slow k.sub.off, as determined during epitope mapping studies. K.sub.d for the four scFv studied ranged between 7.3.times.10.sup.-8 and 1.1.times.10.sup.-9 M (Table 5), values comparable to those reported for monoclonal IgG produced from hybridomas (Foote, et al., Nature 352:530-532 (1991)). C25 has the highest affinity (K.sub.d=1.1.times.10.sup.-9 M) reported for an anti-botulinum toxin antibody. k.sub.on differed over 84-fold, and k.sub.off differed over 33-fold, between scFv (Table 5). In vitro toxin neutralization was determined by using a mouse hemidiaphragm preparation and measuring the time to 50% twitch tension reduction for BoNT/A alone and in the presence of 2.0.times.10.sup.-8 M scFv. Values are reported in time to 50% twitch reduction. scFv binding to epitope 1 (S25) and epitope 2 (C25) significantly prolonged the time to neuroparalysis: 1.5-fold (152%) and 2.7-fold (270%), respectively (Table 5 and FIG. 3). In contrast, scFv binding to epitopes 3 and 4 had no significant effect on the time to neuroparalysis. A mixture of S25 and C25 had a significant additive effect on the time to neuroparalysis, with the time to 50% twitch reduction increasing 3.9-fold (390%).

Previous Doc Next Doc Go to Doc#

1: Eur J Biochem. 1994 Jan 15;219(1-2):161-9.

Antagonism of the intracellular action of botul neurotoxin type A with monoclonal antibodies t to light-chain epitopes.

Cenci Di Bello I, Poulain B, Shone CC, Tauc L, Dolly

Department of Biochemistry, Imperial College of Scienc Technology & Medicine, London, England.

mAbs were produced in mice against highly purified, ren chain (LC) of botulinum neurotoxin A (BoNT A) that was immobilised on nitrocellulose to avoid the undesirable us toxoids. Subcutaneous implants of relatively high amoun micrograms each) of LC allowed its slow release into the circulation and, thus, yielded much higher antibody titre the underivatized antigen than had hitherto been obtain conventional immunization. Seven stable hybridoma cell established which secrete mAb of IgG1 and IgG2b subc reactive specifically with BoNT A and LC, in native and d states, without showing any cross-reactivity with types tetanus toxin. The pronounced reactivities of three mA refolded LC or intact toxin, observed in immunobinding a precipitation assays, relative to that seen in Western b preference for conformational epitopes. Though mAbs 4

preference for conformational epitopes. Though mAbs 4 failed to neutralize the lethality of BoNT in vivo, admin intraneurally of mAb7 prevented the inhibition of trans release normally induced by subsequent extracellular ad of BoNT A. Notably, the latter mAb reacted with a synt peptide corresponding to amino acids 28-53 in the N-te the LC, a highly conserved region in Clostridial neurotox reported to be essential for maintaining the tertiary st the chain. Most importantly, when mAbs 4 or 7 were mic inside ganglionic neurons of Aplysia, each reversed, thou transiently, the blockade of acetylcholine release by th novel finding is discussed in relation to the nature of th zinc-dependent protease activity of the toxin.

PMID: 7508383 [PubMed - indexed for MEDLINE]

Aug

11447222 PMID: 11553596

Epitope mapping **of neutralizing** botulinum **neurotoxin A antibodies by phage display.**

Mullaney B P; Pallavicini M G; Marks J D

Department of Laboratory Medicine, University of California at San Francisco, San Francisco, California 94143, USA. mullaney@cc.ucsf.edu

Infection and immunity (United States) Oct 2001, 69 (10) p6511-4, ISSN 0019-9567 Journal Code: 0246127

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Single-chain antibodies neutralize activity and bind nonoverlapping epitopes of botulinum A neurotoxin. Two phage display epitope libraries were constructed from the 1.3 kb of binding domain cDNA. The minimal epitopes selected against the single-chain Fv-Fc antibodies correspond to conformational epitopes with amino acid residues 1115 to 1223 (S25), 1131 to 1264 (3D12), and 889 to 1294 (C25).

Tags: Human; Support, U.S. Gov't, Non-P.H.S.

Descriptors: *Antibodies, Bacterial--immunology--IM; *Botulinum Toxin Type A--immunology--IM; *Clostridium botulinum--immunology--IM; *Epitopes, B-Lymphocyte--immunology--IM; *Immunoglobulin Fragments--immunology--IM; *Immunoglobulin Variable Region--immunology--IM; Animals; Botulinum Toxin Type A--chemistry--CH; Botulinum Toxin Type A--genetics--GE; Epitope Mapping--methods--MT; Epitopes, B-Lymphocyte--chemistry--CH; Epitopes, B-Lymphocyte--genetics--GE; Mice; Models, Molecular; Neutralization Tests; Peptide Library; Protein Structure, Tertiary

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Botulinum Toxin Type A); 0 (Epitopes, B-Lymphocyte); 0 (Immunoglobulin Fragments); 0 (Immunoglobulin Variable Region); 0 (Peptide Library); 0 (immunoglobulin Fv)

Record Date Created: 20010912

Record Date Completed: 20011025

tous phage: methods for displaying antibody (Fab) heavy and light chains. Nucleic Acids Res. **19**:4133–4137.
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Editor: J. D. Clements

WEST Search History

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☐	L4	L3 and toxin	9
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END OF SEARCH HISTORY

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17199994
Basic Patent (No,Kind,Date): US 6287566 BA 20010911   <No. of Patents: 002>
  Protective peptides neurotoxin of C. botulinum (English)
Patent Assignee: US ARMY (US)
Author (Inventor): DERTZBAUGH MARK T (US)
National Class: *424190100; 424192100; 424239100; 530300000; 530350000;
    930200000
IPC: *A61K-039/00; A61K-039/02; A61K-039/08
Language of Document: English
Patent Family:
    Patent No    Kind  Date      Applic No   Kind  Date
    US 20030185850  AA  20031002    US 917791       A   20010731
    US 6287566      BA  20010911    US 446114       A   19950519  (BASIC)
Priority Data (No,Kind,Date):
    US 917791 A  20010731
    US 446114 A2 19950519
    US 446114 A  19950519

Dialog File: Inpadoc/Fam.& Legal Stat_1968-2004/UD=200436


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16522525
Basic Patent (No,Kind,Date): CA 2336587 AA 20000120   <No. of Patents: 028>
  BOTULINUM NEUROTOXIN VACCINE (English; French)
Patent Assignee: U S MEDICAL RES INST OF INFECT (US)
Author (Inventor): PUSHKO PETER (US); LEE JOHN S (US); SMITH JONATHAN F
    (US); PARKER MICHAEL (US); SMITH LEONARD (US); DERTZBAUGH MARK T (US)
IPC: *C12N-015/09; A61K-048/00; C12N-007/00; C12P-021/00; C12N-015/31
CA Abstract No: *132(09)106948Z; 132(09)106949A; 132(10)121456F
Derwent WPI Acc No: *C 00-160826; C 00-160827; C 00-182165
Language of Document: English
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Priority Data (No,Kind,Date):
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    US 133870 P  19990512
    WO 99US15570 W  19990709
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05725278 PMID: 6171518

Homogeneity and heterogeneity of toxins produced by Clostridium botulinum type C and D strains.

Oguma K; Syuto B; Agui T; Iida H; Kubo S

Infection and immunity (UNITED STATES) Nov 1981, 34 (2) p382-8, ISSN 0019-9567 Journal Code: 0246127

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Tags: Comparative Study; Support, Non-U.S. Gov't

Descriptors: Antigens, Bacterial--analysis--AN; * **Botulinum Toxins** --immunology--IM; * **Clostridium botulinum** --classification--CL; **Botulinum Toxins** --isolation and purification--IP; **Clostridium botulinum** --immunology--IM; Cross Reactions; **Epitopes** ; Immunodiffusion; Neutralization Tests

CAS Registry No.: 0 (Antigens, Bacterial); 0 (Botulinum Toxins); 0 (Epitopes)

Record Date Created: 19820222

Record Date Completed: 19820222

07707190 PMID: 2450068

Establishment of a monoclonal antibody recognizing an antigenic site common to Clostridium botulinum type B, C1, D, and E toxins and tetanus toxin.

Tsuzuki K; Yokosawa N; Syuto B; Ohishi I; Fujii N; Kimura K; Oguma K
Department of Microbiology, Sapporo Medical College, Japan.

Infection and immunity (UNITED STATES) Apr 1988, 56 (4) p898-902, ISSN 0019-9567 Journal Code: 0246127
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

The partial amino acid sequence of the light-chain (Lc) component of Clostridium botulinum type C1 toxin was determined. The sequence was quite similar to those of the other types of botulinum and tetanus toxins. Nine monoclonal antibodies against botulinum type E toxin were established by immunizing BALB/c mice with type E toxoid or its Lc component. Six antibodies reacted with the heavy-chain component and three reacted with the Lc component of the toxin. One of the latter three antibodies reacted with botulinum type B, C1, and D toxins and tetanus toxin, as well as botulinum type E toxin. This antibody recognized the Lc components of these toxins, indicating that there exists one common antigenic determinant on the Lc regions of these toxins.

Tags: Comparative Study; Support, Non-U.S. Gov't
Descriptors: Antibodies, Monoclonal--immunology--IM; *Bacterial Toxins --immunology--IM; * **Botulinum Toxins** --immunology--IM; * **Clostridium botulinum** --immunology--IM; *Tetanus Toxin--immunology--IM; Amino Acid Sequence; Clostridium perfringens--immunology--IM; **Epitopes** ; Immunosorbent Techniques; Molecular Sequence Data
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Bacterial Toxins); 0 (Botulinum Toxins); 0 (Epitopes); 0 (Tetanus Toxin)
Record Date Created: 19880419
Record Date Completed: 19880419

07132287 PMID: 2423459

The use of monoclonal antibodies to analyze the structure of Clostridium botulinum type E derivative toxin.

Kozaki S; Kamata Y; Nagai T; Ogasawara J; Sakaguchi G

Infection and immunity (UNITED STATES) Jun 1986, 52 (3) p786-91, ISSN 0019-9567 Journal Code: 0246127

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Six monoclonal antibodies against Clostridium botulinum type E derivative toxin were prepared. Three of the five binding to the heavy chain neutralized the derivative toxin; the other one binding to the light chain did not. Immunoblotting analysis with the monoclonal antibodies showed that the fragment obtained by tryptic digestion consisted of the light chain and part of the heavy chain (H-1 fragment) linked together by a disulfide bond(s) and that the antigenic determinants common between type E and F derivative toxins were located on both the heavy and light chains. The fragment induced by chymotrypsin treatment, like the tryptic fragment, bound to four monoclonal antibodies. The mild tryptic treatment and reduction resulted in separation of the chymotryptic fragment into two smaller fragments corresponding to the light chain and H-1 fragment. These results indicate that H-1 fragment contains the amino-terminal portion of the heavy chain. The monoclonal antibody neutralizing the toxin and probably recognizing the **epitope** on the carboxyl-terminal portion (H-2 fragment) of the heavy chain effectively competed for binding of 125I-labeled derivative toxin to synaptosomes. Of the two monoclonal antibodies neutralizing the toxin and recognizing the **epitopes** on H-1 fragment, one partially inhibited binding, but the other did not. This suggests that the binding of 125I-labeled derivative toxin depends mainly on the carboxyl-terminal region of the heavy chain and that interference with binding is not the only means of toxin neutralization.

Descriptors: **Botulinum Toxins** --immunology--IM; * **Clostridium botulinum** --immunology--IM; Animals; Antibodies, Bacterial--immunology--IM; Antibodies, Monoclonal--immunology--IM; Antibody Specificity; Antigens, Bacterial--immunology--IM; Chymotrypsin--metabolism--ME; **Epitopes** ; Immunosorbent Techniques; Mice; Neutralization Tests; Synaptosomes --metabolism--ME; Trypsin--metabolism--ME

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Antigens, Bacterial); 0 (Botulinum Toxins); 0 (Epitopes)

Enzyme No.: EC 3.4.21.1 (Chymotrypsin); EC 3.4.21.4 (Trypsin)

Record Date Created: 19860707

Record Date Completed: 19860707

09973574 PMID: 7508383

Antagonism of the intracellular action of botulinum neurotoxin type A with monoclonal antibodies that map to light-chain epitopes .

Cenci Di Bello I; Poulain B; Shone C C; Tauc L; Dolly J O

Department of Biochemistry, Imperial College of Science, Technology & Medicine, London, England.

European journal of biochemistry / FEBS (GERMANY) Jan 15 1994, 219 (1-2) p161-9, ISSN 0014-2956 Journal Code: 0107600

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

mAbs were produced in mice against highly purified, renatured light chain (LC) of botulinum neurotoxin A (BoNT A) that was immobilised on nitrocellulose to avoid the undesirable use of toxoids. Subcutaneous implants of relatively high amounts (up to 10 micrograms each) of LC allowed its slow release into the systemic circulation and, thus, yielded much higher antibody titres against the underivatized antigen than had hitherto been obtained by conventional immunization. Seven stable hybridoma cell lines were established which secrete mAb of IgG1 and IgG2b subclasses reactive specifically with BoNT A and LC, in native and denatured states, without showing any cross-reactivity with types B, E, F or tetanus toxin. The pronounced reactivities of three mAbs towards refolded LC or intact toxin, observed in immunobinding and precipitation assays, relative to that seen in Western blots imply a preference for conformational **epitopes** . Though mAbs 4, 5 and 7 failed to neutralize the lethality of BoNT in vivo, administration intraneurally of mAb7 prevented the inhibition of transmitter release normally induced by subsequent extracellular administration of BoNT A. Notably, the latter mAb reacted with a synthetic peptide corresponding to amino acids 28-53 in the N-terminus of the LC, a highly conserved region in Clostridial neurotoxins reported to be essential for maintaining the tertiary structure of the chain. Most importantly, when mAbs 4 or 7 were microinjected inside ganglionic neurons of Aplysia, each reversed, though transiently, the blockade of acetylcholine release by the toxin; this novel finding is discussed in relation to the nature of the zinc-dependent protease activity of the toxin.

Tags: In Vitro; Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: Antibodies, Monoclonal--pharmacology--PD; * **Botulinum Toxins** --antagonists and inhibitors--AI; * **Botulinum Toxins** --immunology--IM; *Neurons--drug effects--DE; *Neurotoxins--antagonists and inhibitors--AI; Amino Acid Sequence; Animals; Antibodies, Monoclonal--metabolism--ME; Aplysia; Enzyme-Linked Immunosorbent Assay; **Epitopes** --metabolism--ME; Mice; Mice, Inbred BALB C--immunology--IM; Multiple Myeloma; Neurons --physiology--PH; Neurotoxins--immunology--IM; Peptides--chemical synthesis --CS; Peptides--immunology--IM; Tumor Cells, Cultured

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins); 0 (Epitopes); 0 (Neurotoxins); 0 (Peptides)

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Use of monoclonal antibodies as probes for the structure and biological activity of botulinum neurotoxin.

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Experiments were done to help clarify the structure-function relationships that govern the interaction between botulinum neurotoxin and the cholinergic neuromuscular junction. Work was done with type E toxin in three different states: 1) unactivated (post-translational product before proteolytic processing), 2) activated (proteolytically modified product) and 3) denatured. Four different monoclonal antibodies were studied (E3, E14, E17 and E32), three of which were capable of diminishing the potency of the toxin. All four antibodies had approximately equivalent affinity for the unactivated and the activated forms of the toxin. Monoclonals E17 and E32 had little ability to interact with denatured toxin, suggesting they recognized conformational **epitopes** ; monoclonals E3 and E14 retained partial ability to bind to denatured toxin, suggesting they recognized both conformational and linear determinants. When phrenic nerve-hemidiaphragm preparations were exposed to toxin under conditions that allowed binding but retarded internalization, the toxin remained accessible to antibodies. However, when tissues were stimulated in an effort to promote endocytosis, the toxin disappeared from accessibility to antibodies. The data indicate that various antigenic domains remain exposed after binding and suggest that certain parts of the toxin molecule undergo little or no conformational change during binding. The data further indicate that the molecular domains recognized by E14, E17 and E32 are internalized simultaneously.

Tags: In Vitro; Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: Antibodies, Monoclonal--diagnostic use--DU; * **Botulinum Toxins** --pharmacology--PD; Animals; Antibodies, Monoclonal--immunology--IM; **Botulinum Toxins** --chemistry--CH; **Botulinum Toxins** --metabolism--ME; Enzyme-Linked Immunosorbent Assay; Mice; Mice, Inbred BALB C; Neuromuscular Junction--drug effects--DE; Neuromuscular Junction--metabolism--ME; Protein Conformation; Structure-Activity Relationship

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Botulinum Toxins)

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